In Vitro Propagation of Resurrection Plant *Selaginella pulvinata* Using Frond Tips as Explants

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Abstract. The resurrection plant *Selaginella pulvinata* (Hook. & Grev.) Maxim is used as an ornamental and medicinal plant. It is also a good candidate for exploring the desiccation tolerance of resurrection plants. However, there is not an efficient propagation method for *S. pulvinata*. In the present study, we evaluated the establishment of in vitro propagation of *S. pulvinata* using frond tips as explants. The original shoot induction, adventitious shoot proliferation and plantlet growth media, and substrate type of plantlet acclimatization were investigated. The highest induction rate of original shoots ($61.77 \pm 5.17\%$) was obtained on half-strength (1/2) MS medium supplemented with $0.1 \text{ mg} \cdot \text{L}^{-1}$ N⁶-benzylaminopurine (BAP). The 1/2 MS with $1.0 \text{ mg} \cdot \text{L}^{-1}$ BAP was the most effective medium for the adventitious shoot proliferation. The quarter-strength (1/4) MS containing 0.1% (w/v) active charcoal (AC) was optimum for plantlets proliferated from adventitious shoots and plantlet growth. Approximately 98 plantlets could be obtained from one single original shoot via one-time shoot proliferation cultivation and plantlet cultivation. The acclimated plants on a 5:1 (v/v) mixture of peat and perlite had the highest survival rate ($92.13 \pm 1.67\%$). The acclimated plants maintained excellent resurrection ability.

Selaginella P. Beauv. (Selaginellaceae), an ancient and distinctive group, is the largest genus of seed-free vascular plants, with an

estimated 700 to 800 species that exploit a diverse array of the arctic, temperate, tropical, and semi-arid habitats (Arrigo et al., 2013; Banks, 2009; Jermy, 1986; Singh et al., 2019; Zhou et al., 2016). Some species of *Selaginella* are resurrection plants, such as *S. tamariscina* (Wang et al., 2010; Xu et al., 2018), *S. lepidophylla* (Pampurova et al., 2014; Rafsanjani et al., 2015; Yobi et al., 2013), *S. bryopteris* (Deeba et al., 2016), *S. arizonica*, *S. eremophila*, and *S. rupicola* of the North American southwestern deserts (Yu et al., 2017a). With the remarkable vegetative desiccation tolerance, the resurrection plants are able to survive nearly complete anhydrobiosis (<10% relative water content) during prolonged drought

events and resume normal growth when water is available (VanBuren et al., 2018). The phenomenon that the dry and visually “dead” plants come alive after rewetting is fascinating to plant biologists and the lay public (Xiao et al., 2015), thus making resurrection plants a special group of ornamental plants. Interestingly, the nuclear genomes of *Selaginella* are some of the smallest among green plants (Baniaga et al., 2016; Little et al., 2007; Obermayer et al., 2002). Therefore, the resurrection species of *Selaginella* are good candidates for exploring the mechanisms of desiccation tolerance with genomic-based approaches (VanBuren et al., 2018).

Selaginella pulvinata (Hook. & Grev.) Maxim, a typical resurrection plant mainly distributed in exposed limestone areas, has varied usefulness in China (Zhang and Zhang, 2004). It is renowned as an ornamental plant because of its magical resurrection ability, and it is an important medicinal plant listed in Chinese Pharmacopoeia (Chinese Pharmacopoeia Committee, 2015). *S. pulvinata* is used in traditional Chinese medicine for the treatment of traumatic injury and asthma (Cao et al., 2010). Pharmacological investigations revealed its various biological activities, such as anti-cancer (Wang et al., 2016) and anti-inflammatory effects (Huang et al., 2017). Unfortunately, because of its overexploitation, populations of *S. pulvinata* have decreased sharply. Therefore, there is an urgent need to establish the in vitro propagation of *S. pulvinata* to produce high-quality plantlets for horticultural and medicinal use without resorting to harvesting wild populations.

Many fern species have been successfully established in an in vitro propagation system via spores (Barnicoat et al., 2011) and shoot organogenesis from juvenile leaves (Camloha et al., 1994), rhizomes (Winarto and Teixeira da Silva, 2012), callus (Hegde et al., 2006), somatic embryos (Mikuła et al., 2015a, 2015b), and green globular bodies (GGBs) (Amaki and Higuchi, 1991). Among them, the GGB system is regarded as a remarkably efficient method (Higuchi et al., 1987). However, studies of in vitro propagation of fern-ally *Selaginella* are limited. Park et al. (2020) reported in vitro regeneration of nonresurrection species *S. martensii* by using shoot-tips as explants. Because of the different germination times of megaspores and microspores in resurrection species *S. eremophila*, *S. rupicola*, and *S. arizonica*, a two-step in vitro propagation method was used: surface-sterilized megaspores were cultured alone for 3 weeks, followed by the addition of surface-sterilized microspores to the germinated megaspore cultures for co-culture; however, the final fertilization rate was only $\approx 12\%$ in *S. eremophila* and *S. rupicola*, and no fertilization was observed in *S. arizonica* (Yu et al., 2017a). Additionally, there are no reports of in vitro vegetative propagation of resurrection species in *Selaginella*.

We describe an in vitro vegetative propagation protocol for resurrection plant *S. pulvinata* using frond tips as explants. We

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investigated the optimal media for original shoot induction, adventitious shoot proliferation and plantlet growth, and the optimal substrate type for plantlet acclimatization. This new protocol will be beneficial for horticultural and medicinal applications of *S. pulvinata* and will be a critical tool for researching the biology of desiccation tolerance.

Materials and Methods

Plant material. *S. pulvinata* plants (plant diameter, 5.0–8.0 cm) were collected from exposed limestone in the Xishan Mountains, Yunnan Province, China (lat. 24°57'6" N, long. 102°38'22" E). Plants were cultivated in plastic pots containing a humus soil obtained from the original habitat and irrigated with tap water every 5 d; they were maintained in a greenhouse for 2 months with natural light [photosynthetic photon flux density (PPFD) 1600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 12:00 PM] and relative humidity $\approx 50\%$ to 60% at $25 \pm 5^\circ\text{C}$.

Induction of original shoots and proliferation of adventitious shoots. Tips of juvenile fronds (length, 0.8–1.2 cm) used as explants (Fig. 1A) were cleaned with running tap water for 2 h; then, they were surface-sterilized for 15 min with 0.1% (w/v) HgCl_2 solution. Afterward, they were rinsed five times with sterile distilled water. Finally, surface-sterilized frond tips were inoculated on 1/2 MS media (Murashige and Skoog, 1962) that contained cytokinin, 3% (w/v) sucrose, and 0.7% (w/v) plant agar and adjusted to pH 5.8 before being autoclaved. Using plant growth regulator (PGR)-free 1/2 MS medium as a control, the effects of cytokinin on original shoot induction were evaluated at different concentrations of BAP

(0.1, 0.5, or 1.0 $\text{mg}\cdot\text{L}^{-1}$) and Thidiazuron (TDZ) (0.1, 0.5, or 1.0 $\text{mg}\cdot\text{L}^{-1}$). Cultures were maintained in the dark at $25 \pm 2^\circ\text{C}$. After 6 weeks of culture, the induction rate of the original shoots was recorded. The original shoots were the shoots derived from the apical and lateral bud primordium. The explant forming at least one original shoot was identified as successful induction.

For the proliferation of adventitious shoots, the original shoots induced on 1/2 MS with 0.1 $\text{mg}\cdot\text{L}^{-1}$ BAP were separated from explants and subsequently cultivated on 1/2 MS media with various concentrations of cytokinin (described previously); then, they were maintained in a controlled environment room at $25 \pm 2^\circ\text{C}$ under light intensity of 40 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ (16 h light/8 h dark) provided by cool-white fluorescent lamps (Philips, Netherlands). After 8 weeks of culture, the number of adventitious shoots proliferated from the single original shoot was assessed.

Plantlet cultivation and acclimatization. To obtain plantlets from adventitious shoots, the single adventitious shoot (height ≥ 5.0 mm) from 1/2 MS supplemented with 1.0 $\text{mg}\cdot\text{L}^{-1}$ BAP was cultivated on PGR-free media consisting of different mineral salt concentrations (1/4 MS, 1/2 MS, or MS) and 0.1% (w/v) AC or no AC. Cultures were incubated in a controlled environment room (as described previously). After 10 weeks of culture, the number of plantlets proliferated from one single adventitious shoot, root length, and frond number of plantlet were recorded.

To choose the optimal substrate type for plantlet acclimatization, the rooted plantlets (height ≥ 1.0 cm) were gently washed in 0.100% to 0.125% (w/v) chlorothalonil solution and planted in plastic pots (diameter, 10 cm) containing different substrate types [i.e.,

peat and a mixture of peat and perlite at 5:1 and 8:1 (v/v)]. All substrate type treatments included two groups: one group had 2.0 $\text{kg}\cdot\text{m}^{-3}$ granular slow-release fertilizer (nitrogen, 14%; phosphorus, 14%; potassium, 14%; w/w) added and the other group did not. These plantlets were maintained in the shaded greenhouse (PPFD, 170 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 12:00 PM) at $20 \pm 5^\circ\text{C}$. Spray irrigation was performed every 7 to 9 d for 4 weeks. Afterward, the shade cloth of the greenhouse was removed, and the PPFD was ≈ 1600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at 12:00 PM. Plantlets were irrigated with tap water every 5 to 6 d. The survival rate of plantlet acclimatization was recorded after 4 weeks of culture in the unshaded greenhouse.

Resurrection assessment of acclimated plants. Hydrated plants cultivated in the unshaded greenhouse for 8 months were used to assess the resurrection ability in a controlled environment room (as described previously). Hydrated plants were not irrigated for more than 10 d to ensure complete dehydration. The dehydrated plants were sequentially kept dry for 1 week and then rehydrated for 24 h. The resurrection rate was recorded by the number of plants recovering to normal growth divided by the total number of plants. At the same time, the relative water content (RWC) of hydrated, dehydrated, and rehydrated plants were measured according to the records of Rapparini et al. (2015).

The chlorophyll fluorescence parameters of hydrated, dehydrated, and rehydrated plants were tested by Chlorophyll Fluorescence Imager (Technologica Inc., Essex, UK). The maximum photosystem II (PSII) efficiency at open centers (F_v/F_m) was collected after 30 min of dark adaption. Non-photochemical fluorescence quenching (NPQ) and maximum PSII efficiency at open centers under illumination ($F_v'F_m'$) were collected after 5 min of light adaption under actinic light (PPFD 600 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$).

Statistical analysis. Experiments were performed in a completely randomized design and repeated three times. Plantlet acclimatization experiments were conducted with three replicates, with each containing 50 plantlets. Other experiments were conducted with six replicates, with each containing five individuals. Data were analyzed by means of an analysis of variance, and the mean comparison was performed using the least significant difference and SPSS 16.0 for Windows (SPSS Inc., Chicago, IL). Significance was set at the 0.05 level.

Results and Discussion

Effects of cytokinin on the original shoot induction and adventitious shoot proliferation. Cytokinin is often used for GGB induction in fern plants (Amaki and Higuchi, 1991; Higuchi and Amaki, 1989; Yu et al., 2017b) and adventitious shoot induction in other seed plants (Alawaadh et al., 2020). Although *S. pulvinata* belongs to fern allies, GGBs were not induced by cultivating the sterilized frond tips on the media containing various

Fig. 1. In vitro propagation of resurrection plant *Selaginella pulvinata*. (A) Frond tip. (B) The induction of original shoots on half-strength (1/2) MS medium supplemented with 0.1 $\text{mg}\cdot\text{L}^{-1}$ N⁶-benzylaminopurine (BAP) after 6 weeks of dark culture. The arrow indicates the original shoot. (C) The proliferation of adventitious shoots on 1/2 MS medium supplemented with 1.0 $\text{mg}\cdot\text{L}^{-1}$ BAP after 8 weeks of culture. (D) Abnormal color of the plantlets cultivated on 1/2 MS medium supplemented with 0.1 $\text{mg}\cdot\text{L}^{-1}$ Thidiazuron (TDZ) after 8 weeks of culture. (E) Plantlets cultivated on quarter-strength (1/4) MS medium containing 0.1% (w/v) active charcoal (AC) after 10 weeks of culture. (F) The acclimated plants after 8 months of culture in the unshaded greenhouse.

concentrations of BAP or TDZ. Therefore, in vitro propagation via GGB should be a unique propagation system in fern species (Higuchi et al., 1987), but not in *S. pulvinata*. However, the adventitious shoots of *S. pulvinata* were obtained through original shoots in our study.

We tested the effects of cytokinin on the original shoot induction and adventitious shoot proliferation using PGR-free 1/2 MS medium as a control. After 6 weeks, explants in all treatments turned brown, and the original shoots derived from the apical and lateral bud primordium (Fig. 1B) occurred in all cytokinin treatments except the control. Table 1 indicates that the original shoot induction was highly dependent on the presence of cytokinin, and that the maximum of induction rate ($61.77 \pm 5.17\%$) was obtained on 1/2 MS medium containing $0.1 \text{ mg}\cdot\text{L}^{-1}$ BAP.

In contrast to sunflower (*Helianthus annuus*) (Zhang and Finer, 2015) and coral tree (*Erythrina variegata*) (Javed et al., 2019), the adventitious shoots of *S. pulvinata* were not directly induced from explants; instead, they proliferated from the original shoots separated from explants and subsequently cultured on 1/2 MS media containing cytokinin. The most efficient proliferation of adventitious shoots was observed on 1/2 MS medium containing $1.0 \text{ mg}\cdot\text{L}^{-1}$ BAP (Table 1, Fig. 1C); on average, 8.33 ± 0.83 adventitious shoots were proliferated from each original shoot. TDZ is an efficient cytokinin for GGB proliferation in fern plant *Cibotium barometz* and adventitious shoot induction in seed plants, such as *Erythrina variegata* (Javed et al., 2019), *Fraxinus nigra* (Lee and Pijut, 2017), and *Scutellaria bornmuelleri* (Gharari et al., 2019). Nevertheless, the addition of TDZ, especially high concentrations (0.5 and $1.0 \text{ mg}\cdot\text{L}^{-1}$), easily resulted in the abnormal color of adventitious shoots in *S. pulvinata* (Fig. 1E), which was consistent with general expectations that TDZ could be related to morphological, physiological, and cytogenetic abnormalities in vitro (Dewir et al., 2018).

Effects of mineral salt concentration and AC on plantlet growth. To obtain plantlets, single adventitious shoots were cultivated on PGR-free media. The effects of the mineral salt concentration and AC on plantlet growth were investigated (Table 2). Although all media were without PGR, several plantlets proliferated from one single adventitious

shoot in all treatments after 10 weeks of culture (Fig. 1C). Similarly, for *Punica granatum*, a PGR-free medium containing $300 \text{ mg}\cdot\text{L}^{-1}$ AC was found to effectively promote shoot multiplication during cultivation of microshoots from previous proliferation medium supplemented with $9.0 \text{ }\mu\text{M}$ BAP (Verma et al., 2020). This phenomenon might be related to the residual cytokinin BAP in the single adventitious shoots of *S. pulvinata* or microshoots of *Punica granatum* from the previous proliferation cultivation.

The mineral salt concentration and AC are associated with plantlet regeneration (Teng, 1997; da Silva et al., 2020). For example, 1/2 MS was optimal for normal sporophyte development from somatic embryos in *Cyathea delgadai* (Mikuła et al., 2015b), but MS favored sporophyte development in *Dryopteris affinis* ssp. *affinis* (Fernández et al., 1996). The 1/4 MS medium with 0.1% or 0.2% AC promoted the plantlets regenerated from GGBs in *Cibotium barometz* (Yu et al., 2017b). Our results also confirmed that the low mineral salt concentration and addition of AC were beneficial for plantlet formation in *S. pulvinata*, and that the largest number of plantlets (11.80 ± 1.28) was obtained from single adventitious shoots cultivated on 1/4 MS medium supplemented with 0.1% (w/v) AC (Table 2). However, the mineral salt concentration and AC had no statistically significant effect on the frond number of plantlets of *S. pulvinata* (Table 2).

Moreover, a low mineral salt concentration and addition of AC were beneficial to the root growth of *S. pulvinata*, and the roots of plantlets cultivated on 1/4 MS medium with 0.1% (w/v) AC ($1.66 \pm 0.11 \text{ cm}$) were significantly longer than those of other treatments (Table 2). Similarly, the addition of 0.1% (w/v) AC improved the root number and length of *Magnolia* 'Ann' (Parris et al., 2012), and 1/4 MS medium with 1.0% (w/v) AC was the recorded optimum for the root development of fern plant *Matteuccia struthiopteris* (Thakur et al., 1998). In contrast, MS without AC was the most efficient for root development of plantlets in nonresurrection species *Selaginella martensii* (Park et al., 2020).

Plantlet acclimatization. The survival rate of plantlet acclimatization for all treatments was above 78% (Table 3). Surviving plants showed a normal phenotype (Fig. 1F). The addition of slow-release fertilizer had no significant effect on the survival rate (Table 3). In particular, the plantlets in plastic

pots containing a 5:1 (v/v) mixture of peat and perlite achieved the maximum survival rate ($92.13 \pm 1.67\%$) (Table 3); therefore, it was considered the optimal substrate type for plantlet acclimatization. The same type of substrate was also suitable for the plantlet acclimatization of *Cibotium barometz* (Yu et al., 2017b). Nevertheless, a 3:1 mixture of horticultural substrate and decomposed granite favored the growth of acclimated plantlets in *Selaginella martensii* (Park et al., 2020).

Resurrection capacity of acclimated plants. In resurrection species, such as *Selaginella lepidophylla* and *Boea hygrometrica*, stem or leaf curling is a morphological mechanism limiting photoinhibitory and thermal damage that the plant might experience in arid environments (Rafsanjani et al., 2015; Xiao et al., 2015). In the study, all the acclimated plants used for testing (Fig. 2A) were tightly curled to form a rough sphere after dehydration (Fig. 2B) and recovered to the normal morphology after 24 h of rehydration (Fig. 2C). The resurrection rate was 100% .

The RWC and chlorophyll fluorescence of acclimated plants in *S. pulvinata* significantly changed with the water conditions (Figs. 3 and 4). When dehydrated plants (RWC $6.86 \pm 1.04\%$) (Fig. 4) were rehydrated for 24 h, the RWC increased to $88.72 \pm 1.98\%$ (Fig. 4), which was closer to the RWC of hydrated plants ($94.20 \pm 0.70\%$) (Fig. 4). At the same time, compared with the hydrated plants, the F_v/F_m , NPQ, and F_v'/F_m' of dehydrated plants sharply decreased to 0.25 ± 0.01 , 0.22 ± 0.02 , and 0.24 ± 0.00 , respectively. Then, these chlorophyll fluorescence parameters recovered significantly after 24 h of rehydration (Fig. 4). The changes in chlorophyll fluorescence parameters indicated that photosynthesis was inhibited under dehydration and revived under rehydration. A similar tendency of RWC and F_v/F_m was also observed in resurrection plants *Selaginella bryopteris* (Pandey et al., 2010), *Selaginella tamariscina* (Xu et al., 2018), *Haberlea rhodopensis* (Rapparini et al., 2015), and *Boea hygrometrica* (Xiao et al., 2015).

Considering the resurrection rate and variable tendency of morphology, RWC, and photosynthesis, the acclimated plants of *S. pulvinata* maintained an excellent resurrection capacity. Therefore, the acclimated plants of *S. pulvinata* would be appropriate for studying the desiccation tolerance of resurrection plants.

Table 1. Effects of cytokinin on the original shoot induction and adventitious shoot proliferation in *Selaginella pulvinata*.

Cytokinin ($\text{mg}\cdot\text{L}^{-1}$)	Original shoot induction rate (%) ^a	No. of proliferated adventitious shoots per original shoot
0	0.00	—
0.1 BA	$61.77 \pm 5.17 \text{ a}$	$2.77 \pm 0.40 \text{ bc}$
0.5 BA	$24.17 \pm 3.57 \text{ bc}$	$3.22 \pm 0.93 \text{ bc}$
1.0 BA	$8.50 \pm 3.84 \text{ c}$	$8.33 \pm 0.83 \text{ a}$
0.1 TDZ	$34.00 \pm 7.48 \text{ b}$	$4.08 \pm 0.92 \text{ b}$
0.5 TDZ	$24.00 \pm 7.48 \text{ bc}$	$3.73 \pm 0.55 \text{ b}$
1.0 TDZ	$22.67 \pm 4.40 \text{ bc}$	$1.63 \pm 0.26 \text{ c}$

^aOriginal shoot induction rate = (number of explants forming original shoot / total number of explants) $\times 100\%$.

Data are illustrated with mean $\pm$ SE (n = 6). Values followed by the same lowercase letter in each column indicate that the means do not differ significantly according to the least significant difference test at the 5% level.

Table 2. Effects of mineral salt concentration and activated charcoal (AC) on plantlet growth in *Selaginella pulvinata*.

Mineral salt concn	AC (% w/v)	No. of proliferated plantlets per adventitious shoot	Root length (cm)	Frond no. per plantlet
1/4 MS	0	4.80 ± 0.58 bc	1.34 ± 0.09 ab	5.40 ± 0.75 a
1/4 MS	0.1	11.80 ± 1.28 a	1.66 ± 0.11 a	6.20 ± 0.11 a
1/2 MS	0	6.60 ± 0.40 b	1.10 ± 0.04 b	5.80 ± 0.73 a
1/2 MS	0.1	6.20 ± 1.53 b	1.06 ± 0.09 bc	6.20 ± 0.37 a
MS	0	4.20 ± 0.58 bc	1.00 ± 0.05 bc	5.80 ± 0.37 a
MS	0.1	2.20 ± 0.37 c	0.76 ± 0.22 c	5.00 ± 0.45 a

Data are illustrated with mean ± SE (n = 6). Values followed by the same lowercase letter in each column indicate that the means do not differ significantly according to least significant difference test at the 5% level.

Table 3. Effects of the substrate type and slow-release fertilizer on the survival rate of plantlet acclimatization in *Selaginella pulvinata*.

Planting substrate (v/v)	Slow-release fertilizer (kg/m ³)	Survival rate of plantlet acclimatization (%) ²
Peat	0	81.94 ± 0.12 bc
Peat	2	79.17 ± 2.12 c
Peat/perlite (5:1)	0	92.13 ± 1.67 a
Peat/perlite (5:1)	2	85.19 ± 2.58 b
Peat/perlite (8:1)	0	85.19 ± 1.23 b
Peat/perlite (8:1)	2	78.87 ± 0.93 c

²Survival rate of plantlet acclimatization = (number of survival plantlets / total number of plantlets for acclimatization) × 100%.

Data are illustrated with mean ± SE (n = 3). Values followed by the same lowercase letter in each column indicate that the means do not differ significantly according to least significant difference test at the 5% level.

Fig. 2. The resurrection ability of the acclimated plants in *Selaginella pulvinata*. (A) Hydrated plant. (B) Dehydrated plant. (C) Rehydrated plant. Bar = 1 cm.

Fig. 3. Changes in chlorophyll fluorescence imaging of the maximum photosystem II (PSII) efficiency at open centers (Fv/Fm) during dehydration and rehydration of acclimated plants in *Selaginella pulvinata*. (A) Hydrated plant. (B) Dehydrated plant. (C) Rehydrated plant.

Conclusion

In the present study, we established an efficient in vitro propagation protocol for *S. pulvinata* using frond tips as explants. Original shoots were efficiently induced on 1/2 MS medium with 0.1 mg·L⁻¹ BAP by using frond tips. Approximately 8.33 adventitious shoots were proliferated from one original shoot on 1/2 MS medium containing 1.0 mg·L⁻¹ BAP, which is the optimal medium for adventitious shoot proliferation. Subsequently, ≈11.80 plantlets were obtained from one adventitious shoot on the optimal plant growth medium 1/4 MS supplemented with

0.1% (w/v) AC. Therefore, ≈98 plantlets could be proliferated from one single original shoot within 18 weeks. The appropriate substrate type for plantlet acclimatization was a 5:1 (v/v) mixture of peat and perlite, and the survival rate of plantlets was more than 92%. A period of approximately 14 months was required from explants to mature plants via the in vitro propagation protocol. Moreover, the acclimated plants maintained excellent resurrection ability. This protocol described could be used to facilitate the rapid propagation of the resurrection plant *S. pulvinata* for horticultural and medicinal purposes. It could

Fig. 4. Changes in the relative water content (RWC) and the chlorophyll fluorescence parameters of acclimated plants in *Selaginella pulvinata* during dehydration and rehydration.

also be a critical tool for researching the biology of desiccation tolerance.

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